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# Assessment of Aflatoxin $M_1$ in Raw Milk of some dairy animals

Nagah Saad[1], Wallaa Amin[2], Zakaria Zaky[3], Lobna Blall[4]
1,2 Department of Food Hygiene, Faculty of Vet. Medicine, Assiut University, Egypt
3 Department of Forensic Medicine and Toxicology, Faculty of Vet. Medicine, Assiut University, Egypt
4 Department of Food Hygiene, Assiut Animal Health Research Institute, Egypt
bosbosadel_78@yahoo.com, wallaa_800@yahoo.com, zakaria@aun.edu.eg, lobna.rabie87@yahoo.com

**ABSTRACT:**
The incidence of aflatoxins $M_1$ was determined in raw milk of cows, sheep and camels. Samples were analyzed by competitive ELISA technique. AFM1 was found in 63.33% of all tested samples by a mean concentration of 23.38±2.26 µg /l. The incidence of $AFM_1$ in raw cow, sheep and camel milk samples were 62.5%, 62.5% and 65%, respectively. The concentration of $AFM_1$ in raw milk was compared to the maximum tolerance limit accepted by the European union/Codex Alimentarius Commission (50 µg/L). The relation between $AFM_1$ contamination in milk samples and different seasons was described. For all lactating species, the incidence of $AFM_1$ was higher in cold season than in hot seasons. Most of tested raw milk samples were contaminated with $AFM_1$ in variable levels with highest $AFM_1$ concentration level in raw cow milk samples. The results indicated that the contamination of milk samples with $AFM_1$ in such levels could be a serious public health problem.

**Keywords:** $AFM_1$, raw milk, seasons, ELISA

## INTRODUCTION

Aflatoxins are carcinogenic metabolites produced by certain molds; *A. flavus*, *A. parasiticus* and *A. nomius* [1]. Certain types of cancer were found to be associated with the exposure to aflatoxins, which led to global concern over food safety [2]. They are highly toxic compounds and can cause both acute and chronic toxicity. Milk is a way for its entrance into the human body. The presence of aflatoxin $M_1$ ($AFM_1$) in milk is a hazard [3]. It is formed by the metabolism of $AFB_1$ in the animal's body following ingestion of contaminated feeds [4]. Although the toxicity of $AFM_1$ is less than that of its parent compound ($AFB_1$), it is known to be hepatotoxic and carcinogenic [5]. IARC, International Agency for Research on Cancer [6] initially classified $AFM_1$ as a group 2B human carcinogen, but IARC [7] moved $AFM_1$ to group 1.

The concentration of $AFM_1$ in milk depends on the amount of ingested $AFB_1$ [8]. Approximately 1-3% of $AFB_1$ in animal feedstuff appears in milk of dairy cow as $AFM_1$. This carryover rate varies from one animal to another, day to day and one milking to another [9]. As for ewes, the carryover rate ranges from 0.60 to 0.72% with a maximum of 2.7% [10]. Aflatoxin $M_1$ could be detected in milk 12-24 hours after the first $AFB_1$ ingestion, reaching a high level after a few days, and then decreases to an undetectable level after 72 hours when the intake of $AFB_1$ is stopped [11].

Numerous studies worldwide have been undertaken to assess the presence of $AFM_1$ in cow's milk [12, 13, 14, 15], sheep milk [16, 17, 18, 19] and camel milk [14, 20, 21].

Due to the harmful effect of $AFM_1$ in milk and milk products, various sets put acceptable limits for $AFM_1$ concentration in milk and its products. Codex Alimentarius Commission [22] and European Commission Regulation [23] prescribed that the maximum limit of $AFM_1$ in liquid milk and milk products is 50 µg/L. However, according to US regulations the level of $AFM_1$ in milk should not be higher than 500 µg/L [24]. In Austria and Switzerland, the maximum level is even lower at 10 µg/L for infant food commodities [25]. In Egypt, the Ministry of Health established that fluid milk and dairy products should be free from $AFM_1$ [26].

Therefore, the present study was directed to detect the contamination of raw milk of some lactating species with $AFM_1$ via different seasons to declare the possible harm for

human health through consumption of such milk.

## MATERIALS AND METHODS

### Collection of samples

A total of 120 raw milk samples collected from dairy farms and dairy shops during the period of October 2013 to August 2014. The samples were 40 raw milk samples; 10 raw milk samples for each season (autumn, winter, spring and summer) from each animal species (Cow, Sheep and Camel). Samples were collected in plastic bottles. Then, samples were transported at 2-4°C in the ice box. Milk samples were stored in deep freezing at -22°C till analysis with competitive Enzyme Linked Immunosorbent Assay [27].

### Analysis of AFM1 by competitive ELISA [28]

The quantitative analysis of AFM1 in milk samples was performed by commercially available competitive ELISA kits (Diagnostic Automation, Inc. Aflatoxin M1 ELISA) according to the manufacturer's instructions.

### Statistical analysis

The statistical program Graph pad Prism 5 (version 5.01) was used for data analysis (Prism 5, 2007). Then described statistics of ANOVA was performed to measure the mean ± standard deviation (SD), the range (minimum to maximum) and the percentage of positive samples for raw milk samples [29].

## RESULTS

**Table 1. Occurrence of $AFM_1$ in the examined raw milk samples**

| Species | No. of examined samples | Positive samples | | Exceeding EC regulations (50μg/L) | | Exceeding Egyptian regulation (0μg/L) | | Mean± SD |
|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | |
| Cow | 40 | 25 | 62.5% | 5 | 20% | 25 | 100% | 30.06±4.925 |
| Sheep | 40 | 25 | 62.5% | 2 | 8% | 25 | 100% | 28.19±2.974 |
| Camel | 40 | 26 | 65% | 1 | 3.85% | 26 | 100% | 12.43±2.737 |
| Total | 120 | 76 | 63.33% | 8 | 10.53% | 76 | 100% | 23.38±2.26 |

**Table 2. Seasonal variation of $AFM_1$ incidence in the examined raw milk samples**

| Season | No. of examined samples | Positive samples | | Exceeding EC regulations (50μg/L) | | Exceeding Egyptian regulation (0μg/L) | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % |
| Autumn | 30 | 30 | 100% | 3 | 10% | 30 | 100% |
| Winter | 30 | 27 | 90% | 2 | 7.40% | 27 | 100% |
| Spring | 30 | 8 | 26.67% | 2 | 22.20% | 8 | 100% |
| Summer | 30 | 11 | 36.70% | 1 | 9.10% | 11 | 100% |
| Total | 120 | 76 | 63.33% | 8 | 10.53% | 76 | 100% |

## DISCUSSION

Aflatoxins have been considered as an important sanitary problem, as it has been demonstrated that the human exposure to mycotoxins may result from consumption of food that are contaminated with toxins and their metabolites [30]. It has been well documented that chronic aflatoxin exposure causes Hepatocellular Carcinoma (HCC), which is the sixth most prevalent cancer worldwide. That's the reason for the International Agency for Research on Cancer

(IARC) to recognize aflatoxins as carcinogenic in 1976 [31]. It is worth mentioning that AFM1 is relatively stable during heat treatment like pasteurization of milk or processing into cheese [32].

The results in Table 1 showed that 63.33% of 120 tested raw milk samples were contaminated with AFM1 with a mean level of 23.38±2.26 µg/L. This incidence of contamination was higher than that of Motawee et al. [33] in Ismailia, Egypt. In contrast, Ghareeb et al. [34] in Qena, Egypt got higher incidence of AFM1. This may be due to the differences of temperature and humidity between north Egypt and south Egypt (Qena and Assiut). In south Egypt where higher temperature and humidity were found which was a favorable condition for growth of fungi and AFB1 production in animal feed stuffs. Higher results were also reported by Gizachew et al. [35] and Abdolgader et al. [36]. Moreover, 10.53% of all positive raw milk samples contained AFM1 in levels above the EC maximum permissible limit (50 µg/L), while all positive samples were above the Egyptian regulation limit (0 µg/L).

Also, the present results revealed that the incidence of AFM1 contamination is 62.5, 62.5 and 65% of the tested raw milk samples of cow, sheep and camel respectively. While, 20%, 8% and 3.85% of positive samples exceeded the maximum permissible limit (50 µg/L) according to European Commission [23] in cow, sheep and camel, respectively. Also, 100% of samples exceeded the Egyptian regulation (0 µg/L). Hussain et al. [14] obtained relatively similar results as 20% of cow milk samples exceeded the EC legal value, while none of the contaminated sheep and camel milk samples exceeded the EC legal level for AFM1. Furthermore, higher AFM1 contamination levels were recorded by Ghanem and Orfi [17] who found that percentages of contaminated raw cow and sheep milk exceeding the European tolerance limit [23] were 59% and 23%, respectively. Also, Motawee et al. [33] revealed that 55.4% and 55.6% of the positive cow and camel milk samples exceeded the European tolerance limit [23] (50 µg/L).

Also, the mean level of AFM1 contamination was determined in raw milk of cow, sheep and camel. Results revealed that the mean level of AFM1 contamination were 30.06±4.925 µg/L, 28.19±2.974 µg/L and 12.43±2.737 µg/L of tested raw milk samples of cow, sheep and camel, respectively.

It is obvious from the aforementioned results that AFM1 contamination level in cow is higher than sheep and camel levels. It is important to realize that AFM1 levels in milk are dependent on the level of AFB1 in the consumed food, so the type of feedstuffs used and environmental factors will influence AFM1 levels in milk. So, this variation may be due to the feeding nature of these animal species as camel are mainly fed by grazing as allowed to roam freely on available wild pasture and forage for their feed requirements without any supplemental feeding. But, sheep occupy an intermediate feeding pattern and are released onto pasture for grazing each morning, and then brought back into an enclosed area in the evening for milking, and provided a prepared ration. Whereas, cows are kept in dairy farms and fed on manufactured feedstuffs made of various stored grain products and byproducts of agricultural industry. Also, the use of dry bread for feed of cows in small and traditional herds is usual in Egypt. That is prone to fungal infection and to subsequent contamination with aflatoxins during storage. So, out-pasturing of milking animals is effective to reduce the level of AFM1 concentration in milk [37].

According to the results recorded in Table 2, the range of AFM1 contamination levels varied among different seasons of the year. The incidence of AFM1 contamination in cold seasons 100% and 90% in autumn and winter, respectively were higher than those in hot seasons 26.67% and 36.7% in spring and summer, respectively. While, 10%, 7.40%, 22.20% and 9.10% of positive samples exceeded the maximum permissible limit (50µg/L) according to European Commission [23] in autumn, winter, spring and summer, respectively. Hussain and Anwar [38] in the Punjab province of Pakistan, Tajkarimi et al.

[39] in Iran and Fallah [40] in Kuwait declared that AFM1 contamination levels are higher in cold seasons than in hot seasons. However, Çelik et al. [41] reported that the contamination levels of AFM1 were found more commonly in spring than winter and also those reported by Asi et al. [18] in Punjab, Pakistan.

The level of this mycotoxin in animal feedstuffs is influenced by the type, the time and method of harvesting, temperature and relative humidity of storage facilities [42]. Results of this study could be explained by feeding practices adopted in Egypt. During summer, fresh animal feed is available such as pasture, grass, weeds and green fodder. However, due to shortage or unavailability of fresh green feed during winter, more concentrate feeding based on corn, wheat, and cotton seeds are used in animal farmhouses. Moreover, green fodder and hay preserved as silage under inadequate storage conditions may be infected with toxigenic Aspergillus fungi and aflatoxins may be formed [13, 37, 39, 43, 44]. There is also evidence that milk yield is lower in winter, which means that AFM1 and other components become more concentrated. Meanwhile, Egypt summer temperature isn't favorable for fungus growth or mycotoxins production. So, AFM1 contamination incidence and level in summer is the lowest among other seasons.

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